

Claims

1. A composition for determining the presence or absence of a target molecule comprising a first ribonucleic acid (RNA) molecule, said first RNA molecule binds a target molecule and has
5 the following formula:

5'-A-B-C-D-E-3';

10 wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "C" denotes a section of the RNA molecule having approximately 1 to 10000 nucleotides which section is capable preventing the replication of the first molecule by the RNA replicase, the letter "D" denotes a
15 section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the first RNA molecule, with sections B and D bound to target, is acted upon by the RNA replicase to form a second RNA molecule, said second RNA molecule has the following formula:

20 5'-E'-X-A'-3';

25 wherein, E' is the complement to E, and A' is the complement to A, and the letter "X" denotes the complement of parts of the sections B and D which may be replicated, or the letter denotes the direct bond between sections E' and A', and said second RNA molecule is replicated by the RNA replicase under replicating conditions.

- 30 2. The composition of claim 1 wherein the sections represented by the letters "A" and "E" are selected from the group of sequences consisting of MDV-I RNA, Q-beta RNA microvariant RNA, nanovariant RNA, midivariant RNA, RQ-135 and modifications of such sequences which maintain the ability of the sequences to be replicated by Q-beta replicase.
- 35 3. The composition of claim 1 wherein the RNA replicase is Q-beta replicase.
- 40 4. The composition of claim 1 wherein the sections B and D bind to target through non-nucleic acid base pairing interactions.
5. The composition of claim 1 wherein the sections B and D each have a hybridization sequence of 1-100 nucleotides, said hybridization sequence of section B is adjacent to the section A and forms a hybridization product with a said hybridization sequence of section D, and said hybridization sequence of section D is adjacent section E.
- 45 6. The composition of claim 1 wherein the section C has 1-10,000 nucleotides which sequences define a stop sequence for the RNA replicase.

7. The composition of claim 1 wherein the sections A and E comprise at least one sequence that hybridizes to a third nucleic acid to form a hybridization product which hybridization product can be detected.

5 8. A composition for determining the presence or absence of a target molecule comprising paired RNA molecules having a first RNA molecule and a second RNA molecule, said first RNA molecule binds a target molecule and has the following formula:

10 $5'-A-F-B-3';$

and, said second RNA binds the target and has the following formula:

$5'-D-H-E-3';$

15 wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the letter "F" denotes a section of the RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product with a section H , the letter "H" denotes a section of the RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product 20 with a section F, in the absence of target, the hybridization sequences do not form a stable hybridization product, in the presence of the target, and the formation of a complex between sections B and D with the target, a hybridization product is formed that allows the RNA replicase to replicate sections A and E to form a third RNA molecule, said third RNA molecule 25 has the following formula:

30 $5'-E'-X-A'-3';$

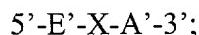
wherein E' is the complement to E and A' is the complement to A, the letter "X" denotes the complement of parts of the sections F and H which may be replicated, or the letter denotes the direct bond between sections E' and A' and said third RNA molecule is replicated by the RNA replicase under replicating conditions.

35 9. A method of determining the presence or absence of a target molecule comprising the steps of:

40 a) providing a first RNA molecule, said first RNA molecule is capable of binding to a target molecule and has the formula:

$5'-A-B-C-D-E-3';$

wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "C" denotes a section of the RNA molecule having approximately 1 to 10000 nucleotides which section is capable preventing the replication of the first molecule by the RNA replicase, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the first RNA molecule, with sections B and D bound to target, is acted upon by the RNA replicase to form a second RNA molecule, said second RNA molecule has the following formula:



wherein, E' is the complement to E, and A' is the complement to A, and the letter "X" denotes the complement of parts of the sections B and D which may be replicated, or the letter denotes the direct bond between sections E' and A', and said second RNA molecule is replicated by the RNA replicase under replicating conditions and combining said first RNA molecule with a sample;

b) imposing binding conditions on a sample potentially containing target molecules in the presence of said first RNA molecule, in the presence of the target molecule, said first RNA molecule forms a target-first RNA molecule complex to form a first modified sample;

c) imposing RNA replicase reaction conditions on the first modified sample, in the presence of an RNA replicase, to form said second RNA molecule in the presence of target to make a second modified sample; and,

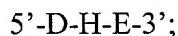
d) monitoring said second modified sample for the presence of the second RNA molecule or its complement, which presence or absence is indicative of the presence or absence of the target molecule.

10.A method of determining the presence or absence of a target molecule comprising the following steps:

a) providing paired RNA molecules comprising a first RNA molecule and a second RNA molecule, said first RNA molecule is capable of binding to a target molecule and has the formula:



said second RNA molecule has the formula:



wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another

sequence D, binds the target molecule under binding conditions, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the letter "F" denotes a section of the
5 RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product with a section H , the letter "H" denotes a section of the RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product with a section F, in the absence of target, the hybridization sequences do not form a stable hybridization product, in the presence of the target, and the formation of a complex between
10 sections B and D with the target, a hybridization product is formed that allows the RNA replicase to replicate sections A and E to form a third RNA molecule, said third RNA molecule has the following formula:

5'-E'-X-A'-3';

15 wherein E' is the complement to E and A' is the complement to A, the letter "X" denotes the complement of parts of the sections F and H which may be replicated, or the letter denotes the direct bond between sections E' and A' and said third RNA molecule is replicated by the RNA replicase under replicating conditions and combining with a sample to form a first sample;
20 b) imposing binding conditions on said first sample potentially containing target molecules in the presence of the first RNA molecule and second RNA molecule, in the presence of the target molecule, the first RNA molecule and the second RNA molecule forms a target-first second RNA molecule complex, to form a second sample;
c) imposing RNA replicase reaction conditions on the second sample, in the presence of an RNA replicase, to form said third RNA molecule in the presence of target, to form a third sample; and
25 d) monitoring said third sample for the presence or absence of said third RNA molecule as an indication of the presence or absence of said target molecule.
30 11. A kit for determining the presence or absence of a target molecule, said kit comprises a one or more reagents comprising a first RNA molecule for use with an RNA replicase, said first RNA molecule has the formula:

5'-A-B-C-D-E-3';

35 wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "C" denotes a section of the RNA molecule having approximately 1 to 10000 nucleotides which section is capable preventing the replication of the first molecule by the RNA replicase, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the first RNA molecule, with sections B

and D bound to target, is acted upon by the RNA replicase to form a second RNA molecule, said second RNA molecule has the following formula:

$$5'-E'-X-A'-3';$$

5 wherein, E' is the complement to E, and A' is the complement to A, and the letter "X" denotes the complement of parts of the sections B and D which may be replicated, or the letter denotes the direct bond between sections E' and A', and said second RNA molecule is replicated by the RNA replicase under replicating conditions, said kit for determining the presence or absence of
10 said target molecule.

12. A kit for determining the presence or absence of a target molecule comprising paired RNA molecules said paired RNA molecules comprising a first RNA molecule and a second RNA
15 molecule, said first RNA molecule has the formula:

$$5'-A-F-B-3'.$$

The second RNA molecule has the formula:

$$5'-D-H-E-3'$$

20 wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in
25 combination, comprise in total at least 10 nucleotides, the letter "F" denotes a section of the RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product with a section H , the letter "H" denotes a section of the RNA molecule having has a hybridization sequence of 1-10,000 nucleotides which form a hybridization product with a section F, in the absence of target, the hybridization sequences do not form a stable
30 hybridization product, in the presence of the target, and the formation of a complex between sections B and D with the target, a hybridization product is formed that allows the RNA
35 replicase to replicate sections A and E to form a third RNA molecule, said third RNA molecule has the following formula:

$$5'-E'-X-A'-3';$$

40 wherein E' is the complement to E and A' is the complement to A, the letter "X" denotes the complement of parts of the sections F and H which may be replicated, or the letter denotes the direct bond between sections E' and A' and said third RNA molecule is replicated by the RNA replicase under replicating conditions.

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13. A method of making a first RNA molecule, wherein the first RNA molecule has the formula:

5 5'-A-B-C-D-E-3'.

wherein A is a section of the RNA molecule having 10-100,000 nucleotides which section is, with another RNA sequence, E, replicated by an RNA replicase, the letter "B" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence D, binds the target molecule under binding conditions, the letter "C" denotes a section of the RNA molecule having approximately 1 to 10000 nucleotides which section is capable preventing the replication of the first molecule by the RNA replicase, the letter "D" denotes a section of the RNA molecule having approximately 1 to 50000 nucleotides which section, with another sequence B, binds the target molecule under binding conditions, the sections B and D, in combination, comprise in total at least 10 nucleotides, the first RNA molecule, with sections B and D bound to target, is acted upon by the RNA replicase to form a second RNA molecule, said second RNA molecule has the following formula:

20 5'-E'-X-A'-3';

wherein, E' is the complement to E, and A' is the complement to A, and the letter "X" denotes the complement of parts of the sections B and D which may be replicated, or the letter denotes the direct bond between sections E' and A', and said second RNA molecule is replicated by the RNA replicase under replicating conditions, comprising the step of combining a sample containing the target molecule with a library of RNA molecules having the formula:

25 5'-A-B'-C-D'-E-3'.

30 to form a mixture of one or more target bound RNA molecules and one or more unbound RNA molecules and the letters B' and D' represent potential sections B and D; adding primer nucleic acid corresponding to at least one section to the mixture with an enzyme capable of degrading the unbound RNA molecules; releasing bound RNA molecules from target; and amplifying said released RNA molecule to form an amplification product to produce RNA molecules having the formula:

35 5'-A-B'-C-D'-E--3'.